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Morphology Rounds at the University of Rochester

Infections Affecting Blood Cell Morphology

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CASE 1. CHRONIC EBV INFECTION

Case Description

A 36-year-old female presented in 1995 with chronic fatigue of four years' duration, evident symptomatically even after 10 hr of sleep. In addition, she noted intermittent epistaxis, gingival bleeding, petechial hemorrhages, and menorrhagia. In 1991, she had transient right cervical lymphadenopathy that resolved quickly and was not biopsied. At that time, the white blood cell count was 14,900/μL with 63% lymphocytes, 27% neutrophils, 10% monocytes; hematocrit was 38% and platelet count was 128,000/μL. A magnetic resonance image (MRI) of the pelvis and abdomen showed small ovarian cysts and an abdominal ultrasound revealed mild splenomegaly. Serology for hepatitis A and B and toxoplasmosis immunoglobulin (Ig)M were negative but the Epstein-Barr virus (EBV) monospot test was positive, and relevant titers showed EBV IgM viral capsid less than 1:10, EBV IgG viral capsid 1:320, EBV early antigen 301 EU (normal, 0–47), and EBV nuclear antigen IgG 1:320. Bone marrow exam was unrevealing. Modest thrombocytopenia, presumed secondary to hypersplenism or immune-mediated destruction, and lymphocytosis persisted over the subsequent four years. Past medical history also included a one-pack-per-day history of cigarette smoking until 1995.

As of 1995, EBV serologies were unchanged from those obtained in 1991. Flow cytometry of peripheral blood mononuclear cells showed a predominance of B lymphocytes with a normal kappa:lambda ratio of surface immunoglobulins, consistent with a polyclonal population.

There was no rearrangement of the heavy chain portion of the immunoglobulin gene and human lymphocyte antigen (HLA)-typing was DR7 positive. Quantitative serum immunoglobulins were not performed. Wright's stain of peripheral blood (Fig. 1a,b) showed mild lymphocytosis, occasional bi-lobed lymphocytes and several very large lymphocytes with abundant cytoplasm and nuclear folding. Red cell morphology was normal but giant platelets were present.

TABLE I. Serial Hemograms*

Date	Total WBC (per μL)	Lymphocytes	Hematocrit	Platelet count
9/91	15,400	63%	36%	128,000
2/92	10,600	—	38%	133,000
6/92	13,700	55%	37%	142,000
8/92	15,100	73%	37%	121,000
12/92	15,600	65%	36%	91,000
9/93	13,600	—	39%	111,000
3/95	5,700	52%	38%	139,000

*WBC, white blood cell.

Comment

Infectious mononucleosis caused by EBV infection occurs in about 500 per 100,000 teenagers each year in the United States, less commonly in individuals over the age of 35 [1]. The classical morphologic features of lymphocytes in patients with EBV infection were described by Downey et al. in 1923 [2]:

1. Type I cells have oval, vacuolated nuclei, open chromatin with blast-like appearance, foamy and vacuolated cytoplasm without granules and intense basophilia at the cytoplasmic edges, which may be indented by adjacent red cells.
2. Type II cells are larger with less nuclear chromatin condensation. The principle cell population in infectious mononucleosis is the Downey type II lymphocyte which expresses CD 8 [3].

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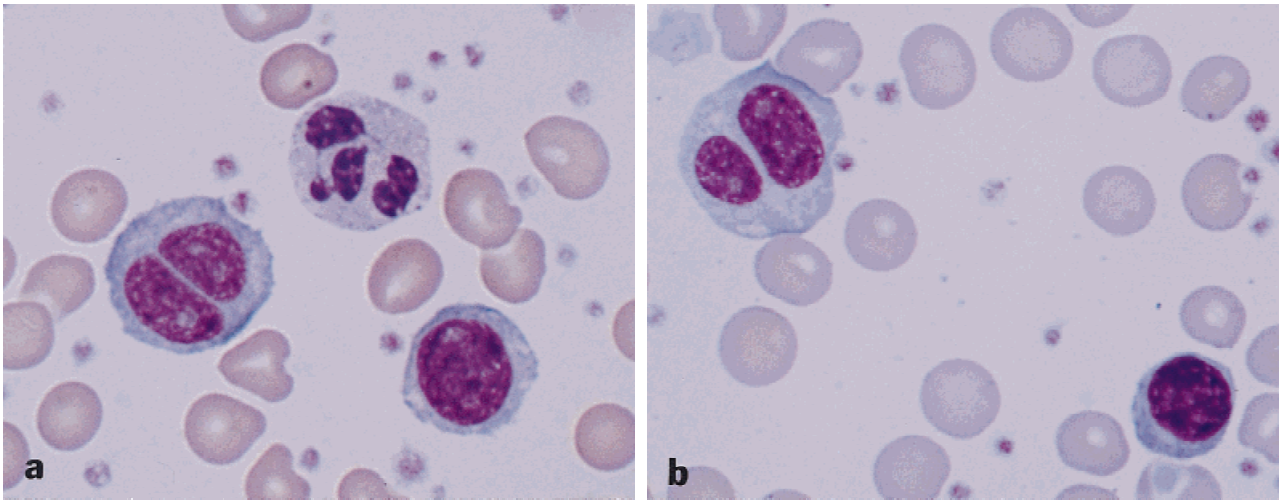

Fig. 1. Chronic B-lymphocytosis with bilobed lymphocytes. a,b: Peripheral blood smear (Wright-Giemsa, ×1,425) showing medium- to large-size lymphoid cells with abundant, basophilic cytoplasm, and characteristic bilobed nuclei. Chromatin is moderately dense and nucleoli can be seen.

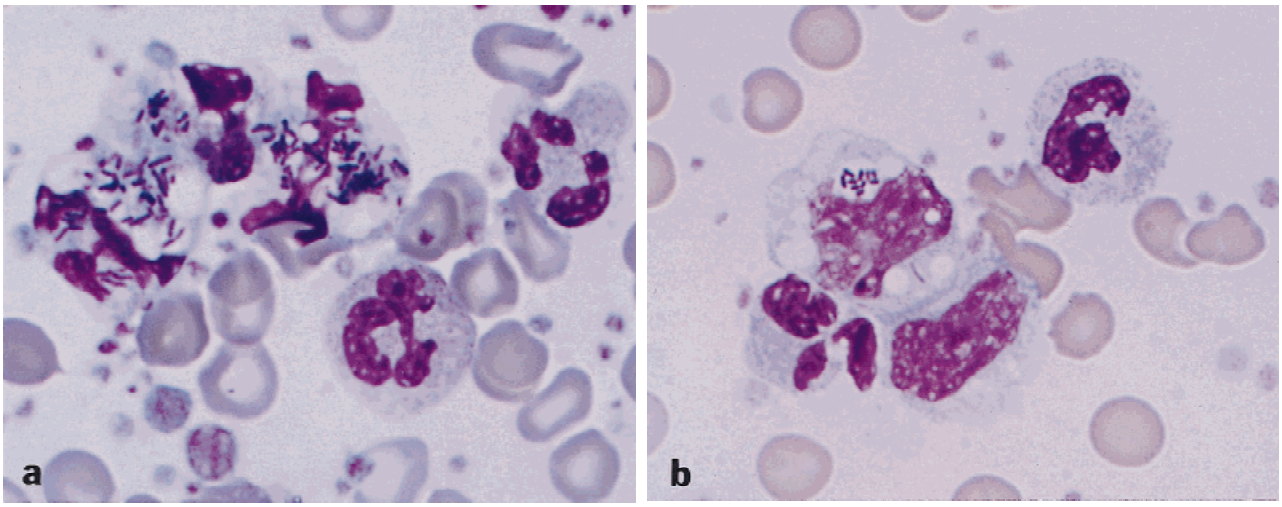

Fig. 2. Catheter-related bacteremia. a,b: Peripheral blood smear (Wright-Giemsa, ×1,425). Polymorphonuclear leukocytes and monocytes showing striking numbers of intracellular microorganisms (coccobacilli-diphtheroids).

3. Type III cells are those that resemble leukemic blast cells. Occasionally the nucleus has a clover-leaf appearance resembling the malignant lymphocytes of adult T-cell leukemia/lymphoma.

Although EBV infects B-lymphocytes, the peripheral blood lymphocytosis usually is comprised of ''reactive'' T-lymphocytes of the suppressor CD8+ phenotype. Typically, this phase is transient, but persistent lymphocytosis in the setting of EBV infection does occur. This is termed ''persistent polyclonal B lymphocytosis'' and is characterized by the following features [4,5,6]:

- Striking female gender predominance
- History of smoking
- Splenomegaly in 38% of patients
- Chronic lymphocytosis with 10–15% bi-lobed lymphocytes

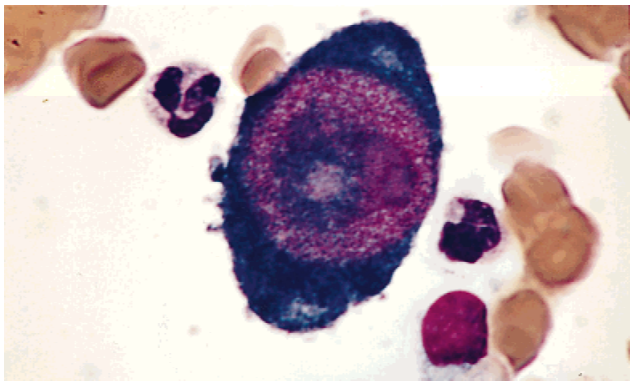

Fig. 3. Parvovirus B19 Infection. Bone marrow aspirate (Wright-Giemsa, ×840) showing a giant pronormoblast with strikingly basophilic cytoplasm and a prominent nucleolus. Intranuclear inclusions are generally not seen in air-dried Wright–Giemsa-stained bone marrow aspirate smears.

- Serology consistent with persistent EBV infection
- Polyclonal increase in IgM (5.0–17.8 g/L) with normal or low IgA and IgG
- Prevalence of HLA DR-7 antigen.

The long-term prognosis of these patients regarding malignant transformation is unknown. Persistent polyclonal B-cell proliferation could be the ''first hit,'' followed in time by additional mutations that could develop into a B-cell lymphoma. A subclone of cells with an additional chromosomal abnormality has been detected in such patients, but the overall indolent and asymptomatic course of patients with chronic B-cell lymphocytosis argues against aggressive therapy [7].

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CASE 2. INTRACELLULAR BACTERIA IN PERIPHERAL BLOOD NEUTROPHILS AND MONOCYTES

Case Description

GS is a 51-year-old white female with multiple myeloma that was initially diagnosed in October 1989 when she presented with a thoracic compression fracture associated with anemia, monoclonal gammopathy, and renal insufficiency. She was treated with melphalan, cyclophosphamide, BCNU, vincristine, and prednisone monthly from December 1989 to April 1991 with a marked reduction in urinary light chain excretion from 10.1 g to 0.3 g. She relapsed and was retreated in August 1992, then underwent high-dose chemotherapy with autologous bone marrow rescue in 1993. After a two-year remission, her disease relapsed in July 1995 and she was treated with cyclophosphamide, 250 mg weekly and prednisone, 100 mg every other day with some decline in her light chain excretion and stabilization of her symptoms. She remained on this regimen until March 1996, when she presented for a routine periodic red cell transfusion and was noted to have an elevated serum creatinine level to 6.0 mg/dl, although she was asymptomatic. An ethylenediaminetetraacetic acid (EDTA) specimen was drawn from the Hickman catheter for a complete blood count (CBC) and differential. Her blood smear revealed striking numbers of coccobacilli within peripheral blood neutrophils and monocytes (Fig. 2a,b). Blood cultures grew Corynebacterium species from both the Hickman port and a peripheral vein. She was treated with vancomycin, the catheter was removed, and the infection cleared.

Comment

The finding of intracellular microorganisms on routine examination of the peripheral blood is unusual, but well described. In the 1940s, preparation of a buffy coat smear was advocated as a useful method for making a rapid diagnosis of bacteremia [1]. However, in 1981, Reik and Rubin [2] found that the threshold concentration of microorganisms that is required to be visible on buffy coat smears is more than 40,000 bacteria/ml of blood, similar to the concentrations of microorganisms that are required to be visible in unspun samples of urine and cerebrospinal fluid. Most adult patients with bacteremia have 10 colony forming U/ml of blood [3], and conditions for visualizing bacteria on peripheral blood smears would usually be present only in patients with overwhelming sepsis. The exception to this rule may exist in immunosuppressed patients with central venous catheters. Routine specimens obtained from such catheters may show intracellular microorganisms when the patient is asymptomatic and not suspected to be bacteremic, although subsequent clinical evidence of sepsis ultimately occurs in most patients [4]. Torlakovic et al. [4] concluded that the organisms seen in catheter-derived blood specimens resulted from direct colonization, as bacteremia persisted until catheter removal. Our case showed organisms in monocytes, as well as in neutrophils, in distinction to their report.

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CASE 3. PARVOVIRUS INFECTION

Case Description

A 38-year-old black male was found to be seropositive for the human immunodeficiency virus two and one half

years prior to his current illness. His CD4 count was 13/μl, and he had prior infections with herpes zoster, oral candidiasis, and genital herpes. He also had chronic renal failure and chronic anemia of mild degree. Current medications were erythropoietin, chlorhexidine topically, nystatin swish and swallow, sodium bicarbonate, and aerosolized pentamidine.

The patient presented to the out-patient clinic with fatigue, weakness, and light-headedness for one week. On physical examination, orthostatic hypotension was noted. There were no palpable lymph nodes or hepatosplenomegaly. The hematocrit was 10%, reticulocyte count was 0.0%, white blood count was 4,300/μl, and platelet count was 270,000/μl. The serum creatinine was 3.3 mg/dl, BUN 37 mg/dl and lactic dehydrogenase 385 mg/dl. A bone marrow examination showed giant proerythroblasts with no evidence of further maturation. Special stains for acid-fast bacilli and fungi were negative and there was no evidence of marrow infiltration with granulomas, lymphoma, or other malignancy. Serum serologies for parvovirus B19 IgM and IgG were negative. Tests to demonstrate parvovirus DNA (polymerase chain reaction [PCR] and DNA hybridization) were not performed. Transfusion of packed red blood cells raised the hematocrit to 20%. Based on the marrow findings (Fig. 3) and the patient's immune deficiency state, a diagnosis of parvovirus-induced pure red cell aplasia was made, and intravenous immune globulin at one gm/kg/day for two days was begun, resulting in a reticulocytosis one week later. There was no evidence for an underlying chronic hemolytic process in this patient after recovery.

Comment

Pure red cell aplasia in the setting of HIV infection can be caused by zidovudine use, Mycobacterium avium intracellulare infection, autoimmune processes, lymphoma/thymoma, and by persistent parvovirus B19 infection [1]. Frickhofen et al. [2] reported in 1990 that persistent B19 parvovirus infection in patients infected with the human immunodeficiency virus (HIV)-1 could be treated with intravenous immunoglobulin. These patients presented with anemia with absent reticulocytes and without fever or rash. Serologic evidence of B19 parvovirus could not be demonstrated in most of these patients, but virus could be detected in serum by PCR and DNA hybridization.

Parvovirus B19 infection in humans is usually acute and self-limited as in so-called ''fifth disease,'' which is associated with skin rash in children and arthralgias in adults. In patients with normal hematopoiesis, infection with parvovirus leads to a transient reticulocytopenia, but any decline in hemoglobin is not noticed due to the brief duration of the infection relative to the life span of circulating erythrocytes. Detectable anemia occurs in the setting of hemolysis and accelerated erythropoiesis, wherein the infection induces a transient aplastic crisis [3], or in immunosuppressed patients, in which case the infection persists due to a lack of antibody response, leading to prolonged suppression of erythropoiesis [4]. Parvovirus generally infects only erythroid precursors, but there have been reports of megakaryocyte abnormalities as well [5].

The ability to clear parvovirus depends on an adequate antibody response, and up to 85% of patients have evidence of previous parvovirus exposure. Patients such as the one presented here with late-stage HIV infection often demonstrate impaired humoral responses, and the administration of pooled immunoglobulin aids in clearing the virus from the blood.

In patients with parvovirus infection, overall marrow cellularity is generally normal. The myeloid/erythroid ratio is increased due to a decrease in erythroid precursors, with a marked paucity of mature erythroid forms. Large proerythroblasts with basophilic cytoplasm and a diffuse immature nuclear chromatin pattern with large nucleoli can be seen, the so-called ''giant pronormoblasts.'' In formalin-fixed bone marrow aspirate smears, intranuclear inclusions, indicative of parvovirus infection, can be seen [6]. The infectious agent DNA can be identified in such cells by in situ hybridization. Recent studies have shown that the RBC P antigen is the receptor for parvovirus entry and that pp individuals are naturally resistant to infection [7].

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